Journal of Natural Products
Vol. 51, No. 6, pp. 1226-1231, Nov-Dec 1988

PLANT ANTIMUTAGENIC AGENTS, 4.[1] ISOLATION AND STRUCTURE ELUCIDATION OF MAESOL, AN INACTIVE CONSTITUENT OF *MAESA* SPP.

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ABSTRACT.—Maesol, a novel dimeric phenol, was isolated from seeds of *Maesa montana* and *Maesa indica*. Maesol was shown to have the formula $C_{28}H_{42}O_4$ with structure **1**, a dimeric, symmetrical 1,12-bis(3,3′-dihydroxy-4,4′-dimethyl-5,5′-dimethoxyphenyl)dodecane. It is the first compound with such structure to be isolated from plant material. Structure elucidation was based largely on ^{1}H- and ^{13}C-nmr techniques and comparison with a known synthetic isomeric dimer **3**. Although crude extracts showed strong inhibition of 2-aminoanthracene activity against *Salmonella typhimurium* (T-98), the pure compound was inactive when tested for inhibition of the mutagenic activity of several mutagens.

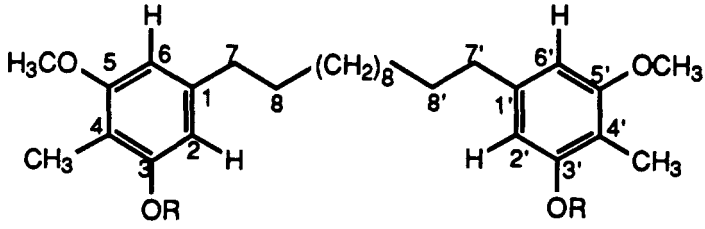
In the course of screening plant extracts for inhibition of the mutagenic activity of 2-aminoanthracene (2AN) toward *Salmonella typhimurium* (1,2), the CH_2Cl_2 fraction from an EtOH extract of the seeds of *Maesa montana* A. DC. (Myrsinaceae), an Indian plant, was found to exhibit confirmed reproducible activity in this assay. The family Myrsinaceae has received extensive phytochemical study by Japanese workers. It has been stated that the presence of alkylated hydroxybenzoquinone derivatives is a chemotaxonomical characteristic of this family (3). Rapanone, embelin, and maesaquinone are among the long chain alkyl 1,4-benzoquinones isolated from *Maesa japonica* and other Myrsinaceae species occurring in Japan [see Ogawa and Natori (3) for review and earlier references]. More recently maesanin, another long chain alkyl substituted 1,4-benzoquinone, has been isolated from an East African plant, *Maesa lanceolata*, used locally as an anticholera medication (4). *Maesa* species from India that have been chemically investigated include *Maesa macrophylla* from which bhogatin, a 1,4-benzoquinone with a 3-nonyl side chain, was isolated (5), *Maesa chisia* (6), and *Maesa indica* (7). From the former, used locally as an insecticide, α-spinasterol and β-amyrin were identified; in the latter sitosterol and quercetin-3-rhamnoside were found.

A literature search yielded no information that *M. montana* had received phytochemical study. Guided by 2AN inhibition assays, flash chromatography yielded an active multicomponent fraction from which a 2AN-inactive, pure compound we have named maesol was isolated as the major component. Maesol is not a benzoquinone. Our studies show that it is a novel dimeric phenol with structure **1**. In this paper we present the isolation and structure elucidation of maesol.

EXPERIMENTAL

MUTAGENIC INHIBITION.—Inhibition of the mutagenic activity of 2AN plus S-9 enzyme toward *S. typhimurium* (T-98) by the crude and purified fractions was determined by procedures described by us in detail in a previous paper (2). A concentration of 2.5 μg/plate of 2AN was used in all cases; initial concentration of test substance was 600 μg, and in some cases (i.e., toxicity or activity determinations) concentrations of 300 and 150 μg were also assayed. The effect of pure maesol was studied on other mutagens; e.g., acetylaminofluorene (AAF), and benzo[*a*]pyrene (B[*a*]P), which require metabolic activation with the Ames S-9 enzyme preparation (8). The concentrations (μg/plate) of AAF and B[*a*]P were 25 and 1.0, respectively. The average numbers of colonies of positive controls (i.e., no test substance present) were 2AN 2500, AAF 743, and B[*a*]P 351. In most cases the colonies were counted after 72 h incubation at 37.5°.

[1]For Part 3 in this series, see Wall *et al.*, *J. Nat. Prod.*, **51**, 1148 (1988).



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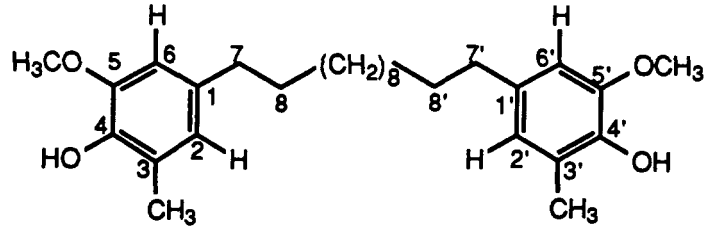
2 R=Ac



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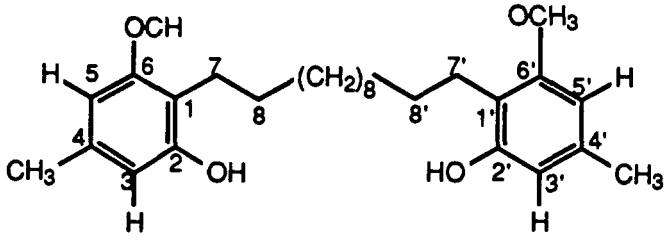

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Toxicity determinations were conducted in the absence of mutagen but presence of histidine, S-9, and test substance (2).

GENERAL ISOLATION AND CHARACTERIZATION PROCEDURES.—Melting points were determined on a Kofler hotstage microscope and are uncorrected. ^{1}H- and ^{13}C-nmr spectra were obtained with a Bruker WM250 spectrometer using TMS as an internal standard and $CDCl_3$ as a solvent. High resolution mass spectra were obtained with an AEI MS-902 instrument. Uv spectra were obtained in MeOH with a Varian 2290-UV-VIS spectrometer, ir spectra with a Perkin-Elmer 467 grating spectrometer. Standard chromatography was carried out on Si gel (E. Merck) 230–240 mesh, or Baker Flash chromatography Si gel using, in general, CH_2Cl_2 as eluent with a gradient of 0.5–10.0% MeOH. For tlc determinations precoated Si gel plates were utilized; normal phase EM precoated Si gel 60, F254, usual solvent 10% MeOH in CH_2Cl_2; reversed phase, Baker precoated Si gel C_{18}-F plates, usual developer 5–10% H_2O in MeOH. Exposure of plates to iodine vapor was used as a general detection agent; alternatively spraying with phosphomolybdate reagent followed by heating was utilized.

PLANT MATERIAL.—The plant material was supplied through the auspices of the Drug Research and Development Branch, National Cancer Institute by the Medicinal Plant Resources Laboratory, Plant Genetics and Germplasm Institute, Agricultural Research Service, USDA, Beltsville, Maryland. A voucher specimen documenting this collection is deposited in the Herbarium of the National Arboretum, Agricultural Research Service, USDA, Washington, D.C. The plant material (seeds) was collected in India in July 1974.

ISOLATION OF MAESOL.—A sample of 300 g of seeds of *M. montana* was extracted by percolation with EtOH at room temperature. After concentration of the EtOH extract in vacuo, the residue was partitioned between CH_2Cl_2 and H_2O. The 2AN assay of the CH_2Cl_2 fraction gave an inhibitory activity (IA) of 44% at a dose of 0.6 mg/plate. After partition between equal volumes of petroleum ether and 90% MeOH/10% H_2O, all the activity was found in the latter (4 g extract), IA = 73% at 0.6 mg. Chromatography over Si gel and elution with C_6H_{14}, then C_6H_{14} containing 10–75% CH_2Cl_2, and then with CH_2Cl_2 and CH_2Cl_2/MeOH 1–10% gave two major fractions weighing 0.75 and 0.77 g with similar tlc patterns.

These fractions demonstrated increased IA, 85% at 0.3 mg. Chromatographic fractions obtained before and after these had low weights and relatively weak inhibitory activity. Crystallization of the combined major fractions from EtOAc/C_6H_{14} gave maesol [**1**] as a white crystalline solid. It had no 2AN inhibitory activity. The same compound was isolated in a similar manner as the major product from the seeds of *M. indica*: mp 76–78°; uv λ max (MeOH) 271 (log ε 3.18), 280 sh (log ε 3.11), after addition of NaOH λ max 281 (log ε 3.45), 288 (log ε 3.46) nm; ir ($CHCl_3$) 3595, 3340 (free and H-bonded OH), 3000 (aromatic CH), 1618, 1591 (aromatic C=C), 1250 (C-O, phenolic OH) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; hrms *m/z* calcd for $C_{28}H_{42}O_4$, 442.3083, found *m/z* 442.3082. *Anal.* calcd for $C_{28}H_{42}O_4$, C 75.97, H 9.56; found C 76.25, H 9.61. R_f = 0.22, E. Merck Si gel KG 60F254, 0.25 mm plate, solvent 1.5% MeOH/$CHCl_3$.

MAESOL DIACETATE [**2**].—Maesol [**1**] was converted to its diacetate by standard methods. The acetate was homogeneous by tlc (1.5% MeOH-$CHCl_3$, Si gel 0.25 mm plate E. Merck, R_f value 0.67): ir ($CHCl_3$) 1750 (O-C-CH_3) cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.55 (s, 2H, H-2,2′), 6.47 (s, 2H, H-6,6′), 3.80 (s, 6H, 2-OMe), 2.55 (t, J = 8 Hz, 4H, 2-ϕ-CH_2), 2.28 (s, 6H, 2-OAc), 1.98 (s, 6H, 2-ϕ-Me), 1.59 (m, 4H, 2-ϕ-CH_2-CH_2-), 1.28 (m, 16H, 2-ϕ-CH_2-CH_2-(CH_2-)$_4$; hrms *m/z* calcd for $C_{32}H_{46}O_6$, 526.3294; found *m/z* 526.3308.

1,12-BIS(4-HYDROXY-5-METHOXY-3-METHYLPHENYL)DODECANE [**3**].—This is a known compound that had been prepared by Schill *et al.* (9): mp 98–99°, uv (MeOH) λ max 281 (log ε 3.65), after adding NaOH 286 (log ε 3.70), 297 sh (log ε 3.59) nm; ir ($CHCl_3$) 3550 (strong H-bonded OH), 3000 sh (aromatic CH), 1620, 1608 (aromatic C=C) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr Table 2; tlc R_f = 0.57, same conditions as for **1** above.

RESULTS AND DISCUSSION

The isolated compound maesol [**1**] was shown to have the molecular formula $C_{28}H_{42}O_4$ both by hrms and C and H analysis. Ir spectroscopy showed absorptions consistent with a nonbonded hydroxyl group and a phenyl ring. The uv absorption at 271 nm which shifted to 280 nm on treatment with NaOH confirmed the presence of a phenolic hydroxyl in maesol. On acetylation, maesol formed a diacetate **2**. Analysis of **2** by hrms gave the molecular formula $C_{32}H_{46}O_6$, confirming the presence of two hydroxyl groups in maesol. The ir spectrum of **2** showed no hydroxyl groups and the presence of a strong carbonyl band at 1750 cm^{-1}. A literature review showed that no compounds of natural origin with the formula $C_{28}H_{42}O_4$ had been reported and that only one compound, a synthetic intermediate 1,12-bis(4-hydroxy-5-methoxy-3-methylphenyl)dodecane [**3**] had been prepared some years ago (9). Comparison of maesol with an authentic reference specimen[2] of **3** indicated both similarities and major differences.

The melting points of maesol and **3** differed by some 20°, and tlc comparisons of maesol and **3** showed that maesol was more polar. In accord with this, the ir spectrum of **3** indicated that the hydroxyl moiety was completely hydrogen bonded, whereas in maesol, the hydroxyl moiety was largely free. This was in accord with the known structure of **3** in which the hydroxyl moieties at positions 4,4′ are within excellent hydrogen bonding range with the 5,5′-methoxy oxygen atoms.

As a working hypothesis we commenced with the assumption that maesol would also be a phenolic dimer but with the OH, OMe, and Me substituents in different positions from those of **3** on the aromatic ring. The structure of **1** was determined unambiguously by a combination of standard and specialized nmr studies.

NMR STUDIES.—Comparison of a ^1H-nmr spectrum of maesol with that of **3** showed that maesol was a dimer. The dimer consisted of a 12-carbon alkyl chain connecting identically substituted aromatic rings, each containing two noncoupled protons, a methoxyl, a hydroxyl, and a methyl substituent (Table 1). ^{13}C-nmr studies supported the proton assignments (Table 2). Based on the spectral data as well as other ex-

[2]We wish to thank Professor G. Schill, Freiburg University for providing us with a generous sample of **3**.

TABLE 1. ^{1}H-nmr Data of Maesol [**1**] and Synthetic Analog **3**.

Proton[a] No.	Maesol [1]				Synthetic Analog 3			
	Shift (δ)	Mult.	*J* (Hz)	Protons	Shift (δ)	Mult.	*J* (Hz)	Protons
H-2	6.30	s		2	6.57	s		2
H-6	6.28	s		2	6.56	s		2
3-OH	4.76	s		2	5.50[b]	s		2
5-OMe	3.80	s		3	3.85	s		3
1-CH_2	2.50	t	7.68	2	2.49	t	7.72	2
4-Me	2.07	s		3	2.22[b]	s		3
1-CH_2-CH_2	1.57	m	7.25	2	1.56	m	7.01	2
1-$(CH_2)_2$-$(CH_2)_4$	1.26	m		16	1.28	m		16

[a]Numbering as shown in structures **1** and **3**. Carbon numbers refer to both identical carbons of the dimer; thus, C-1 = C-1 + C-1′, etc.

[b]In **3**, OH is at C-4 and Me is at C-3.

perimental information obtained from maesol and **3**, a number of isomeric structures were possible for maesol. Structures with adjacent hydroxyl and methoxyl groups were excluded from consideration because of the absence of hydrogen bonding in maesol based on ir and tlc comparisons.

For each proposed structure, ^{13}C shift values were calculated for the aromatic carbons. These values were then compared to experimental data. Of the structures proposed, **1** and **4** gave shift values closest to the experimental data.

Several additional nmr experiments established **1** as the correct structure of maesol. A nondecoupled ^{13}C-nmr spectrum was obtained on the sample with sufficient data point resolution to observe couplings larger than 1 Hz. The nondecoupled spectrum showed long-range coupling of both protonated aromatic carbons on the order of 6 Hz. This coupling could be attributed to 3-bond coupling to the other aromatic proton as well as to either the methyl protons for structure **4** or the methylene protons for structure **1**. Coupling of the methyl carbon would also be expected if the aromatic protons

TABLE 2. ^{13}C-nmr Data of Maesol [**1**] and Synthetic Analog **3**.

Carbon Atom[a]	Maesol [1]			Synthetic Analog 3	
	Shift (δ)	Mult.[b]	*J*[c] (Hz)	Carbon Atom[a]	Shift (δ)
C-1	141.63	s		C-1	133.80
C-2	107.93	d	156.10	C-2	108.32
C-3	154.03	s		C-3	123.23
C-4	109.01	s		C-4	141.42
C-5	158.26	s		C-5	145.94
C-6	103.44	d	156.31	C-6	122.75
5-OCH_3	55.55	q	143.6	5-OCH_3	55.88
1-CH_2-	35.97	t	126.3	1-CH_2-	35.66
1-CH_2-CH_2	31.26	t	128.4	1-CH_2-CH_2	31.84
1-$(CH_2)_2$-$(CH_2)_4$	29.40	t	125.0	1-$(CH_2)_2$-$(CH_2)_4$	29.53
4-CH_3	7.73	q	127.5	3-CH_3	15.28

[a]Numbering as shown in structures **1** and **3**. Carbon numbers refer to both identical carbons of the dimer; thus, C-1 = C-1 + C-1′, etc.

[b]Multiplicities determined by off-resonance ^{13}C nmr.

[c]Coupling constants determined by proton nondecoupled ^{13}C nmr.

were attached to carbons 3 and 5 as in structure **4**. However, the methyl carbon showed no long-range coupling. Confirmation of structure **1** was obtained from a long-range 2D proton-carbon correlation (COLOC) experiment (10) (Figure 1), which effectively eliminated structure **4**. The COLOC experiment showed definite coupling of the protonated aromatic carbons with the benzylic protons of the alkyl chain. All other correlations (Table 3) confirmed structure **1** as the structure of maesol.

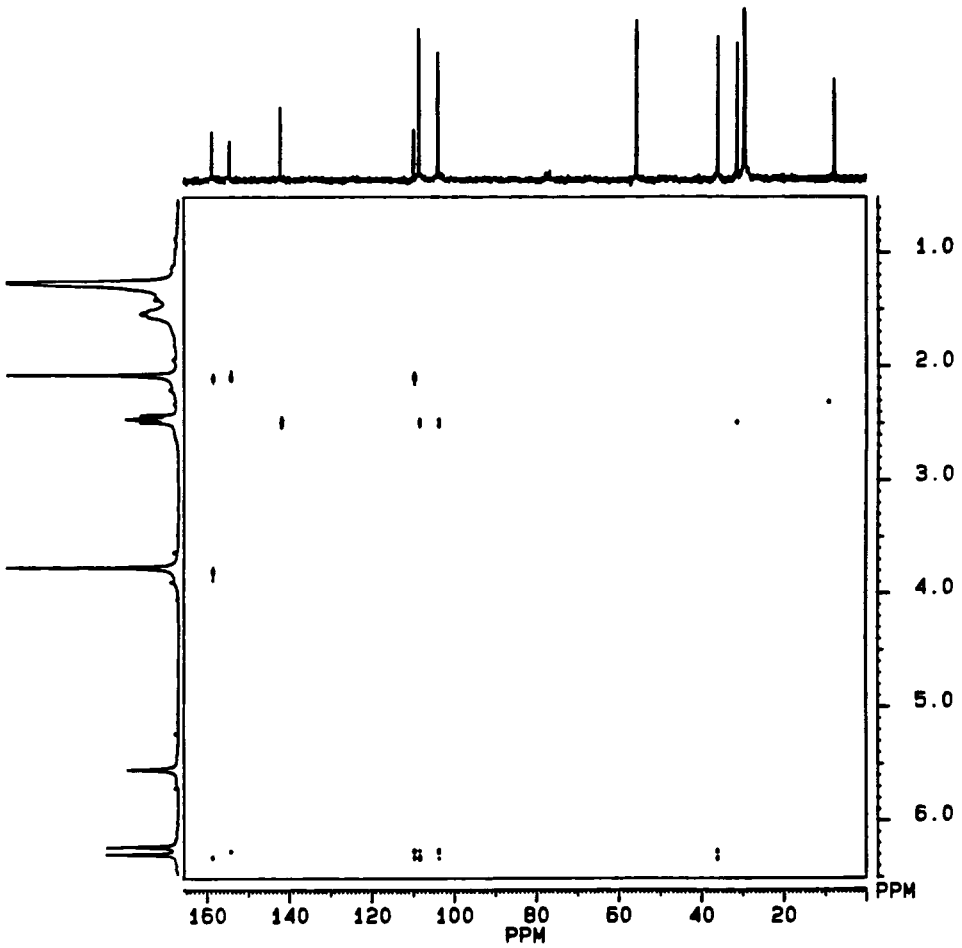

FIGURE 1. Long-range proton carbon correlation (COLOC) of maesol [**1**].

BIOLOGICAL ACTIVITY.—Maesol was inactive at doses of 600 μg/plate in the inhibition of the mutagenic activity of 2AN, AAF, and B[*a*]P toward *S. typhimurium* (T-98) plus S-9. Hence, the inhibitory activity observed in crude extracts of *M. montana* and *M. indica* is evidently due to another plant constituent, the nature of which is under investigation. After our studies were completed, we noted a paper from Hecht's laboratory (11) on DNA scission by several 5-alkylresorcinols obtained from *Hakea trifurcata*. One of these was a phenolic dimer linked by a 14-carbon chain. Maesol was tested for DNA scission by Hecht's procedure (11) but was inactive.

TABLE 3. Long-Range 2D Proton-Carbon Correlation Data for Maesol [1].

Carbon[a]	^{13}C Shift (δ)	^{1}H Correlation	^{1}H Shift (δ)
C-1	141.68	1-CH_2	2.49
C-2	108.20	H-6	6.25
		1-CH_2	2.49
C-3	153.96	4-Me	2.09
C-4	109.49	4-M	2.09
		H-2	6.32
		H-6	6.26
C-5	158.33	5-OMe	3.80
		4-Me	2.09
C-6	103.51	H-2	6.32
		1-CH_2	2.49
1-CH_2	35.91	H-2	6.32
		H-6	6.25

[a]Numbering as shown in structure **1**. Carbon numbers refer to both identical carbons of the dimer; thus, C-1 = C-1 + C-1′, etc.

ACKNOWLEDGMENTS

This research was partially supported by NCI Grant CA 38245-03. We wish to thank Mr. Thomas Hughes and Ms. Janet Warner for antimutagen assays.

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Received 14 June 1988